

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	
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Ming-Bo Wang et al.)	Group Art Unit: 1635
)	
Application No.: 09/287,632)	Examiner: JANE J ZARA
)	
Filed: April 7, 1999)	Confirmation No.: 6526
)	
For: METHODS AND MEANS FOR)	
OBTAINING MODIFIED PHENOTYPES)	
)	
)	

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Peter Robert Schofield of the Prince of Wales Medical Research Institute, Sydney, New South Wales, 2010, Australia, declare as follows:

1. I am currently the Executive Director and CEO of the Prince of Wales Medical Research Institute. I also hold an appointment as a Professor of Medicine at the University of New South Wales.
2. I graduated from the University of Sydney with the degree of Bachelor of Agricultural Science with Honours in 1982. In 1985, I received my PhD in genetics from the Australian National University. In 1998, I was awarded the degree of Doctor of Science by the University of New South Wales. I have published over one hundred and ninety scientific papers in the fields of genetics, molecular biology and neuroscience. I have given presentations at many local and international conferences and serve on several government committees in the healthcare/pharmaceuticals area. A brief form of my CV is attached as Exhibit 1.
3. This declaration relates to an examination by the United States Patent and Trademark Office (USPTO) to United States Patent Application No. 09/287,632 in the name of Commonwealth Scientific and Industrial Research Organisation ("CSIRO").

4. In view of my qualifications and experience in molecular biology, especially my knowledge of gene cloning and gene structure, I believe I am qualified to comment on the technical aspects of the United States Patent Application No. 09/287,632 (hereinafter "the '632 application").
5. I have read and understood the following documents which are relevant to the present examination:
 - a) Complete specification of PCT Patent Application No. WO 99/53050, which is equivalent to the '632 application;
 - b) An Office Action from the USPTO dated February 8, 2007 in connection with the '632 application;
 - c) The pending claims of the '632 application.
6. I have been requested by the CSIRO to provide my comments in relation to the statements made by the USPTO examiner in the Office Action, specifically with regard to claims 85, 91, 106, 107 and 108, which recite the inclusion of an intron.
7. Unless stated to the contrary, when I express an opinion in this Declaration, I am expressing the view that I believe a person of ordinary skill in the field of the '632 application would have held as of 8 April 1998, the priority date of the application.

My Opinion

8. Example 6 of the specification describes the use of an intron (*Flaveria trinervia* pyruvate orthophosphate dikinase intron 2) in a DNA region encoding sense and antisense sequences. Tobacco plants transformed with the constructs were subsequently challenged with PVY. The results presented in Table 8 (page 47) show that the inclusion of an intron (in either the sense or antisense orientation) resulted in 22 or 21 of the 24 independent transgenic plants being immune to PVY challenge, respectively. This surprising result represented the highest percentage of plant resistance to disease reported in the entire specification, indicating that inclusion of an intron provided a highly desirable feature in construct design and treatment efficacy.
9. This is further explained in the specification (page 23) in which it is stated "In fact, the inventors have unexpectedly found that inclusion of an intron sequence in the chimeric DNA genes encoding an RNA molecule comprising the hairpin RNA, preferably in the spacer region, and preferably in the sense orientation, enhances the efficiency of reduction of expression of the target

nucleic acid. The enhancement in efficiency may be expressed as an increase in the frequency of plants wherein silencing occurs, or as an increase in the level of reduction of the phenotypic trait."

10. Example 1 of the specification (page 36) also makes reference to introns. In this case it states "a castor bean catalase intron (Ohta et al., 1990) as modified by Wang et al. (1997) ("intron")." Subsequently it is stated (page 37) "In addition, T-DNA vectors were constructed to evaluate the influence of a presence of an intron sequence in the chimeric genes encoding CoP constructs."
11. The results of these experiments are reported in Example 1 and Table 2. It states (page 39) "Supertransformation with GUSd in a sense or antisense orientation, with or without an intron or an early stop codon, showed some degree of reduction (in about 25% of the analysed calli) of the endogenous GUS activity".
12. Thus, the specification makes reference to introns generally, as discovered and described by Sharp and Roberts, and then proceeds to demonstrate the effects of using two different intron sequences, supporting the view that the inclusion of an intron (any intron) will confer the desirable features of the invention.
13. A person of ordinary skill in the field, would have understood that introns are part of the transcribed DNA sequence of a gene that is non-coding or intervening, and are removed from the heterogeneous nuclear RNA (hnRNA or pre-mRNA) transcript of the gene by the spliceosome complex. The spliced hnRNA becomes the mRNA. Most, but not all, eukaryotic genes contain introns, as do mitochondrial and chloroplast genes.
14. Introns were discovered in 1977 by Phillip Sharp and Richard Roberts. Their discovery altered the previous view that genes were continuous stretches of DNA that served as direct templates for mRNA molecules, which form the templates for protein synthesis. This discovery was recognized by the award of the Nobel Prize for Physiology or Medicine in 1993. The press release, issued by the Nobel Assembly, about this work and its significance is notable because it refers to introns as a class, and does not make specific distinctions about any particular intron. The press release is attached hereto as Exhibit 2.
15. The general nature of this discovery, and its translation to all of eukaryotic cell biology was such that it was featured in both the most highly rated scientific journals such as *Science* and in the

more generally accessible journals such as *Scientific American*. For example, Francis Crick stated in a review in *Science* on "Split genes and RNA splicing" (*Science* 204: 264-271, 1979) that "A number of genes in higher organisms and in their viruses appear to be split. That is, they have "nonsense" stretches of DNA interspersed within the sense DNA. The cell produces a full RNA transcript of this DNA, nonsense and all, and then appears to splice out the nonsense sequences before sending the RNA to the cytoplasm. In this article what is known about these intervening sequences and about the processing of the RNA is outlined. Also discussed is their possible use and how they might have arisen in evolution." Similarly, an article by Pierre Chambon titled "Split Genes" was published in 1981 describing the discovery and characterization of introns (*Scientific American* 244, 60-71).


16. This view about the generality of introns was supported by the research that I undertook, the journal articles that I read and the work conducted by my colleagues in the same laboratory from the commencement of my PhD studies in 1982 and further from the commencement of my postdoctoral studies in 1985.
17. Specific examples of work involving the identification of introns and confirming that all introns conform to a common design was undertaken in my laboratory by my colleagues. For example, in 1982, Dr Robert Richards published an article in the journal *Nature* in which they reported the complete nucleotide sequence of two members of the human metallothionein gene family. They were able to deduce that one gene was a functional metallothionein-II gene, while the other was a pseudogene, lacking introns, but terminating in a poly(A) tail. (Karin, M and Richards, RI. (1982) Human metallothionein genes--primary structure of the metallothionein-II gene and a related processed gene. *Nature* 299: 797-802).
18. In another study undertaken my fellow PhD student, Anthony Mason, he characterized and published an article in the journal *Nature* describing the structure, including intron-exon structure, of a mouse genomic clone containing a complete kallikrein gene (mGK-1) and the 3' end of another (mGK-2). (Mason, AJ, Evans, BA, Cox DR, Shine, J and Richards RI. (1983) Structure of mouse kallikrein gene family suggests a role in specific processing of biologically active peptides. *Nature* 303: 300-307).
19. Other research in my laboratory concerned the cloning of the leghaemoglobin gene from the soybean. Both Marcker's and Verma's laboratories reported the primary gene structures of structures of soybean leghemoglobin genes. It will be noted that the term 'primary structure'

24. The Office Action states (page 4) "The specification, claims and the art do not adequately describe the distinguishing features or attributes concisely shared by the members of the broad genus comprising DNA constructs whereby any intronic sequence is inserted anywhere in the chimeric DNA, and whereby the DNA construct provides for the function claimed, of generating a gene silencing construct that reduces phenotypic expression of any nucleic acid of interest in any plant and in any eukaryotic cell." Further the examiner states "The specification fails to teach or adequately describe a representative number of species in the genus such that the common attributes or characteristics concisely identifying members of the proposed genus are exemplified (e.g. the myriad of sequences encompassed by the genus intron, or intronic sequences is vast, and further whereby any intronic sequence is inserted anywhere within the DNA construct and a DNA chimeric construct generates a gene silencing construct which reduces the phenotypic expression of any nucleic acid of interest in any eukaryotic cell). And because the genus claimed is so highly variant, the description provide is insufficient whereby a representative number of chimeric constructs provide for the functions claimed, of reducing the phenotypic expression of any nucleic acid of interest in any eukaryotic cell or plant. One of skill in the art would reasonably conclude that the disclosure, at the time of filing, fails to provide a representative number of species to describe the broad genus claimed. Thus, Applicant was not in possession of the claimed genus."
25. As noted above, the specification teaches two intron sequences, not one as stated in the Office Action (page 4).
26. With regard to the state of the art, by the early 1980s it was clear that the broad genus 'intron' could be easily and reliably defined by comparison of genomic DNA and cDNA (or mRNA) sequences, and that there were well defined elements that allowed the categorical definition of introns.
27. That the inclusion of an intron significantly enhances the effectiveness of the construct in reducing target gene expression is abundantly clear from the specification of the Application.
28. The Office Action also refers to statements in Smith et al. *Nature* 407: 319-320, 2000. For example, the examiner quotes Smith who state "How does the presence of this intron enhance silencing efficiency? The process of intron excision from the construct by the spliceosome might help align the complementary arms of the hairpin in an environment favouring RNA hybridization, promoting the formation of a duplex. Alternatively, splicing may transiently

increase the amount of hairpin RNA by facilitating, or retarding, the hairpin's passage from the nucleus, or by creating a smaller, less nuclease-sensitive loop."

29. Ultimately, the mechanism by which the presence of an intron results in the enhanced efficiency of target gene silencing is not relevant to what is being claimed in the current claims of the '632 application. A person of ordinary skill in the field of the application would have understood from reading its specification that the presence of an intron (generally any intron) will enhance the target gene silencing. This is supported by the examples in the specification in the light of the prior art literature which clearly specifies and defines the broad genus 'intron.'
30. Given the general applicability of the teachings of the specification to any potential target gene and any intron, it is my opinion that the '632 application sufficiently described the full range of chimeric DNA constructs recited in the claims. A person of ordinary skill would have recognized that the teachings of the specification could be practiced with more than a sufficient number of known nucleic acid sequences of interest (i.e. target genes) and intron sequences to be representative of the genus as a whole. Therefore, I believe that a person of ordinary skill in the filed would have recognized that the applicants were in possession of the full scope of the claimed genus at the time the application was filed.
31. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: OCTOBER 30TH 2007


Peter Robert Schofield

BIOGRAPHICAL SKETCH

Professor Peter R Schofield PhD DSc
Executive Director and Chief Executive Officer, Prince of Wales Medical Research Institute
and Professor of Medicine, University of New South Wales
Barker Street, Randwick, NSW 2031
Sydney, Australia.

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
University of Sydney, Sydney, Australia	BScAgr HonsI	1982	Genetics
Australian National University, Canberra, Australia	PhD	1985	Genetics
Genentech Inc, South San Francisco, CA, USA	postdoctoral	1985-1987	Molecular Neuroscience
Centre for Molecular Biology, University of Heidelberg, Germany	postdoctoral	1987-1988	Molecular Neuroscience
University of New South Wales, Sydney, Australia	DSc	1998	Medicine

A. Positions and Honors

Positions and Employment

1988-1991 Senior Scientist, Pacific Biotechnology Limited, Rushcutters Bay, Sydney
1991-2006 Garvan Institute of Medical Research, Sydney (1991-93, Senior Research Fellow; 1994-98, Principal Research Fellow; 1999-2005, Senior Principal Research Fellow; 2006 Honorary SPRF)
1993-2005 NHMRC Research Fellowship (1993-1996, Senior Research Fellow; 1997-2000, Principal Research Fellow, Senior Principal Research Fellow 2001-2005)
1999-2004 Director, Neurobiology Research Program, Garvan Institute of Medical Research, Sydney
2000-Present Professor, School of Medicine, University of New South Wales, Sydney
2004-Present Executive Director and Chief Executive Officer, Prince of Wales Medical Research Institute, Sydney

Other Experience and Professional Memberships

1992 - 2004 Foundation Director, Secretary (1994-2000) & President (2000-04), Genome Conference, Inc.
1995 - 2000 Secretary (1995-2000) & Chairman (2000-04) Garvan Institute, Institutional Biosafety Committee.
1995 - 2005 Member, Pharmaceutical Subcommittee, Australian Drug Evaluation Committee, A statutory committee providing advice to the Federal Minister for Health & Ageing)
1997 - 2002 Director, President-Elect (2000-01), & President (2001-02), The Australian Society of Medical Research
1998 - 2007 Co-Convenor, The Neuroscience Panel, and Member of Scientific Steering Committee
NISAD, Neuroscience Institute for Schizophrenia and Allied Disorders
2000 - 2004 Member of Steering Committee and Foundation Director, Research Australia Ltd
2003 Member, Prime Minister's Science, Engineering and Innovation Council (PMSEIC) Working Group "Brain and Mind Disorders: Impact of the Neurosciences"
2004 - 2005 Member, National Neuroscience Consultative Task Force, Invited by the Federal Minister of Health
2004 - 2006 Member, Program Committee, 11th International Congress of Human Genetics
2005 Member, Legislation Review Committee, Prohibition of Human Cloning Act 2002 & Research Involving Human Embryos Act 2002. Appointed by the Federal Minister for Ageing
2007 - present Member, Research Council, Schizophrenia Research Institute Australia (formerly NISAD)

Honors

1982 The University Medal, Faculty of Agriculture, Sydney University
1990 A. W. Campbell Award, Australian Neuroscience Society
1991 Elsie Waltham Thompson Award, National Heart Foundation of Australia
1995 Boehringer-Mannheim Medal, Australian Society for Biochemistry and Molecular Biology Inc.
1997 Gottschalk Medal, Australian Academy of Science

B. Selected peer-reviewed publications (in chronological order).

(Publications selected from 173 peer-reviewed publications)

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rhee LM, Rodriguez H, Ramachandran J, Glencorse TA, Reale V, Seeburg PH and Barnard EA (1987) Sequence and functional expression of the GABA_A receptor shows a ligand-gated receptor superfamily. **Nature** 328: 221-227
Levitan ES, Schofield PR, Burt DR, Rhee LM, Wisden W, Koehler M, Fujita N, Rodriguez H, Stephenson FA, Darlison MG, Barnard EA & Seeburg PH (1988) Structural and functional basis for GABA_A receptor heterogeneity. **Nature** 335: 76-79

- Pritchett DB, Sontheimer H, Gorman CM, Kettenmann H, Seeburg PH and Schofield PR (1988) Transient expression shows ligand gating and allosteric potentiation of GABA_A receptor subunits. **Science** 242: 1306-1308
- Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR and Seeburg PH (1989) A novel GABA_A receptor subunit important for benzodiazepine pharmacology. **Nature** 338: 582-585
- Vandenberg RJ, French CR, Barry PH, Shine J and Schofield PR (1992) Antagonism of ligand-gated ion channel receptors: Two domains of the glycine receptor α subunit form the strychnine binding site. **PNAS** 89: 1765-1769
- Vandenberg RJ, Handford CA and Schofield PR (1992) Distinct agonist- and antagonist-binding sites on the glycine receptor. **Neuron** 9: 491-496
- Ryan SG, Buckwaller MS, Lynch JW, Handford CA, Segura L, Shiang R, Wasmuth JJ, Camper SA, Schofield P and O'Connell P (1994) A missense mutation in the gene encoding the $\alpha 1$ subunit of the inhibitory glycine receptor causes the spasmodic mouse phenotype. **Nature Genetics** 7: 131-135
- Rajendra S, Lynch JW, Pierce KD, French CR, Barry PH & Schofield PR (1995) Mutation of a single amino acid in the human glycine receptor transforms β -alanine and taurine from agonists into competitive antagonists. **Neuron** 14: 169-75
- Lynch JW, Rajendra S, Pierce KD, Handford CA, Barry PH & Schofield PR (1997) Identification of intracellular and extracellular domains mediating signal transduction in the inhibitory glycine receptor chloride channel. **EMBO J** 16: 110-120
- Baker M, Kwok JBJ, Kucera S, Crook R, Farrer M, Houlden H, Isaacs A, Lincoln S, Onstead L, Hardy J, Wittenberg L, Dodd P, Webb S, Hayward N, Tannenberg A, Andreadis A, Hallupp M, Schofield PR, Dark F & Hutton M (1997) Localisation of fronto-temporal dementia with Parkinsonism in an Australian kindred to chromosome 17q21-22. **Annals Neurology** 42: 794-798
- Adams LJ, Mitchell PB, Fielder SL, Rosso A, Donald JA and Schofield PR (1998) A susceptibility locus for bipolar affective disorder on chromosome 4q35. **American Journal of Human Genetics** 62: 1084-1091
- Hutton M, *et al.* (1998) Coding and 5'-splice-site mutations in tau with inherited dementia FTDP-17. **Nature** 393: 702-5
- Kwok JBJ, Li Q-X, Hallupp M, Whyte S, Beyreuther K, Masters CM and Schofield PR (2000) Novel Leu723Pro APP mutation increases A β 42(43) peptide levels and induces apoptosis. **Annals of Neurology** 47: 249-253
- Stanford PM, Halliday GM, Brooks WS, Kwok JBJ, Storey CE, Creasey H, Morris JGL, Fulham M and Schofield PR (2000) Progressive supranuclear palsy pathology caused by a novel silent mutation S305S in exon 10 of the tau gene: Expansion of the disease phenotype caused by tau mutations. **Brain** 123: 880-893
- Smith MJ, Kwok JBJ, McLean CA, Kril JJ, Broe GA, Nicholson GA, Cappai R, Hallupp M, Cotton RGH, Masters CL, Schofield PR and Brooks WS (2001) Variable phenotype of Alzheimer's disease with spastic paraparesis. **Annals of Neurology** 49: 125-129
- Lynch JW, Han N-LR, Haddrill J, Pierce KD and Schofield PR (2001) The surface accessibility of the glycine receptor M2-M3 loop is increased in the channel open state. **Journal of Neuroscience** 21: 2589-2599
- Badenhop RF, Moses MJ, Scimone A, Mitchell PB, Ewen KR, Rosso A, Donald JA, Adams LJ and Schofield PR (2001) A genome screen of a large bipolar affective disorder pedigree supports prior evidence for a susceptibility locus on chromosome 13q. **Molecular Psychiatry** 6: 396-403
- Rees MI, Lewis TM, Kwok JBJ, Mortier G, Govaert P, Snell RG, Schofield PR and Owen MJ (2002) Hyperekplexia associated with compound heterozygote mutations in the β -subunit of the human inhibitory glycine receptor (GLRB). **Human Molecular Genetics** 11:853-860
- Badenhop RF, Moses MJ, Scimone A, Mitchell PB, Ewen KR, Rosso A, Donald JA, Adams LJ and Schofield PR (2002) A genome screen of 13 bipolar affective disorder pedigrees provides evidence for susceptibility loci on chromosome 3 as well as chromosomes 9,13 and 19. **Molecular Psychiatry** 7: 594-603
- Blair IP, Adams LJ, Badenhop RF, Moses MJ, Scimone A, Morris JA, Ma L, Austin CP, Donald JA, Mitchell PB and Schofield PR (2002) A transcript map encompassing a susceptibility locus for bipolar affective disorder on chromosome 4q35. **Molecular Psychiatry** 7: 867-873
- Kwok JBJ, Kapoor R, Gotoda T, Iizuka Y, Yamada N, Kato N, Isaacs K, Kushwaha V, Church WB, Schofield PR and Kapoor V (2002) A missense mutation in kynurenine aminotransferase-1 in spontaneously hypertensive rats. **Journal of Biological Chemistry** 277:35779-35782
- Stanford PM, Shepherd CE, Halliday GM, Brooks WS, Schofield PW, Brodaty H, Martins RN, Kwok JBJ and Schofield PR (2003) An increase in three repeat tau causes frontotemporal dementia. **Brain** 126: 814-826
- Kwok JBJ, Hallupp M, Halliday GM, Brooks WS, Dolios G, Murayama O, Badenhop RF, Wang R, Gandy SE, Vickers J, Takashima A and Schofield PR (2003) Presenilin-1 mutation Leu271Val results in altered exon 8 splicing and Alzheimer's disease with non-cored plaques and no neuritic dystrophy. **J Biol Chem** 278: 6748-6754
- Brooks WS, Kwok JBJ, Kril JJ, Broe GA, Blumbergs PC, Tannenberg AE, Lamont PJ, Hedges P and Schofield PR (2003) Alzheimer's disease with spastic paraparesis and "cotton wool" plaques: two pedigrees with PS-1 exon 9 mutations. **Brain** 126: 783-791
- Segurado R, *et al.* (2003) Genome Scan Meta-Analysis of Schizophrenia and Bipolar Disorder Part III: Bipolar Disorder. **American Journal of Human Genetics** 73: 49-62
- Absalom NL, Lewis TM, Kaplan W, Pierce KD and Schofield PR (2003) Role of charged residues in coupling ligand binding and channel activation in the extracellular domain of the glycine receptor. **J Biol Chem** 278: 50151-50157

Kwok JBJ, Teber E, Loy C, Hallupp M, Nicholson G, Mellick GD, Buchanan DD, Silburn PA & Schofield PR (2004) Tau promoter haplotypes regulate transcription and are associated with Parkinson's disease. **Annals Neurology** 55:329-334

Shepherd CE, Gregory GC, Vickers JC, Brooks WS, Kwok JBJ, Schofield PR, Kril JJ and Halliday GM (2004) Positional effects of presenilin-1 mutations on tau phosphorylation in cortical plaques. **Neurobiology of Disease** 15: 115-119

Verdile G, Groth D, Mathews PM, St George-Hyslop P, Fraser PE, Ramabhadran TV, Kwok JBJ, Schofield PR, Carter T, Gandy S and Martins RN (2004) Baculoviruses expressing the human familial Alzheimer's disease presenilin 1 mutation lacking exon 9 increase levels of an amyloid beta-like protein in Sf9 cells. **Molecular Psychiatry** 9: 594-602

Brooks WS, Kwok JBJ, Halliday GM, Godbolt A, Rossor MN, Creasey H, Jones AO and Schofield PR (2004) Hemorrhage is uncommon in new Alzheimer's family with Flemish amyloid precursor protein mutation. **Neurology** 63: 1613-1617

Kwok JBJ, Hallupp M, Loy CT, Chan DKY, Woo J, Mellick GD, Buchanan DD, Silburn PA, Halliday GM and Schofield PR (2005) GSK3B polymorphisms alter transcription and splicing in Parkinson's disease. **Annals of Neurology** 58: 829-839

Halliday GM, Song YJC, Lepar G, Brooks WS, Kwok JBJ, Kersaitis C, Gregory GC, Shepherd CE, Rahimi F, Schofield PR and Kril JJ (2005) Pick bodies in a family with Presenilin-1 Alzheimer's disease. **Annals of Neurology** 57: 139-143

Wilhelm K, Mitchell PB, Niven H, Finch A, Wedgwood L, Scimone A, Blair IP, Parker G and Schofield PR (2006) Life events first depression onset and the serotonin transporter gene. **British Journal of Psychiatry** 188: 210-215

Blair IP, Chetcuti AF, Badenhop RF, Scimone A, Moses MJ, Adams LJ, Craddock N, Green E, Kirov G, Owen MJ, Kwok JBJ, Donald JA, Mitchell PB and Schofield PR (2006) Positional cloning association analysis and expression studies provide convergent evidence that the cadherin gene *FAT* contains a bipolar disorder susceptibility allele. **Molecular Psychiatry** 11: 372-383

Gregory GC, Macdonald V, Schofield PR, Kril JJ and Halliday GM (2006) Differences in regional brain atrophy in genetic forms of Alzheimer's disease. **Neurobiology of Aging** 27: 387-393

Graham BA, Schofield PR, Sah P, Margrie TW & Callister RJ (2006) Distinct physiological mechanisms underlie altered glycinergic synaptic transmission in the murine mutants *spastic spasmodic* and *oscillator*. **J Neuroscience** 26: 4880-90

Pickering-Brown SM, Baker M, Gass J, Boeve BF, Loy CT, Brooks WS, McKenzie IRA, Martins RN, Kwok JBJ, Halliday GM, Kril J, Schofield PR, Mann DMA and Hutton M (2006) Mutations in *progranulin* explain atypical phenotypes with variants in *MAPT*. **Brain** 129: 3124-3126

Karl T, Duffy L, Scimone A, Harvey RP & Schofield PR (2007) Altered motor activity exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. **Genes Brain & Behaviour** (in press)

Boucher AA, Arnold JC, Duffy L, Schofield PR, Micheau J and Karl T (2007) Heterozygous neuregulin1 mice are more sensitive to the behavioural effects of Δ^9 -tetrahydrocannabinol. **Psychopharmacology** 192: 325-336

McAuley EZ, Blair IP, Liu Z, Fullerton JM, Scimone A, van Herten M, Evans MR, Kirkby KC, Donald JA, Mitchell PB and Schofield PR (2007) A genome screen of 35 bipolar affective disorder pedigrees provides significant evidence for a susceptibility locus on chromosome 15q25-26. **Molecular Psychiatry** (in press)

Alexander DM, Williams LM, Gatt JM, Dobson-Stone C, Kuan SA, Todd EG, Schofield PR, Cooper NJ and Gordon E (2007) The contribution of apolipoprotein E alleles on cognitive performance and dynamic neural activity over six decades. **Biological Psychiatry** 75: 229-238

Dobson-Stone C, Gatt JM, Kuan SA, Grieve SM, Gordon E, Williams LM and Schofield PR (2007) Investigation of MCPH1 G37995C and ASPM A44871G polymorphisms and brain size in a healthy cohort. **NeuroImage** 37: 394-400

Karlstrom H, Kwok JBJ, Gregory GC, Hallupp M and Schofield PR (2007) No association of spastic paraparesis genes in PSEN1 Alzheimer's disease with spastic paraparesis. **NeuroReport** 18: 1267-1269.

Sinclair A and Schofield PR (2007) Human embryonic stem cell research: An Australian perspective. **Cell** 128: 221-223

Graham BA, Brichta AM, Schofield PR and Callister RJ (2007) Altered potassium channel function in the superficial dorsal horn of the *spastic* mouse. **The Journal of Physiology** (in press)

Rademakers, R et al. (2007) An international initiative to study phenotypic variability associated with progranulin haploinsufficiency in patients with the common c.1477C>T (p.R493X) mutation. **The Lancet Neurology** (in press)

C. Research Support.

Current Support

NHMRC = Australian National Health and Medical Research Council (NIH equivalent)

ARC = Australian Research Council (NSF equivalent)

NHMRC/ARC "Ageing Well, Ageing Productively" Program Grant No 401162 Sachdev (PI) 1/1/2007-12/31/2011
 "Gene-Environment interactions in healthy brain ageing and age-related neurodegeneration"

Role: Co-investigator

Study: A newly established collaborative study to recruit and longitudinally follow older Australian Twins for markers of healthy ageing and identify gene-environment interactions that lead to neurodegeneration.

JO & JR Wicking Trust

Schofield (PI)

1/1/2007-12/31/2009

"An information and support system for families with hereditary dementia – an Australia-wide program"

Role: PI Study: A pilot grant to provide information and support to Australian early onset Alzheimer's disease APP and PSEN1 pedigree members.

Australian Rotary Health Research Fund - Grant into Alzheimer's Disease Kwok (PI) 1/1-12/31/2007
"The role of genetic contributors in the presentation of psychiatric symptoms in Alzheimer's disease"

Role: Co-Investigator Study: A pilot grant to identify and recruit research participants with Alzheimer's disease and behavioural symptoms through the use of Medicare health records.

ARC Linkage Grant No LP0455104 Williams (PI) 11/1/2004-10/31/2007

"Development of integrated biological markers of brain function"

Role: Co-investigator Study: A collaborative grant working with the startup neuroinformatics group Brain Resource Company to examine and correlate genetic markers with phenotypic markers of normal brain function.

NHMRC Project Grant No 376011 Schofield (PI) 1/1/2006-12/31/2008

"The biological role of the cadherin gene FAT in bipolar disorder susceptibility"

Role: PI Study: To define the role of the first positionally cloned susceptibility gene for bipolar disorder, FAT, and to identify the biological mechanisms by which the disease associated SNPs may cause disease.

NHMRC Enabling Grant No 401184 Schofield (PI) 1/1/2006-12/31/2010

"Genetic Repositories Australia"

Role: PI Study: An 'enabling grant' (research facilities and infrastructure) to establish and operate a national DNA and Cell Repository to facilitate pedigree and population studies.

ARC Discovery Project Grant No DP0774248 Schofield (PI) 1/1/2007-12/31/2009

"Identification of genetic polymorphisms of synaptically expressed genes that contribute to variation in normal brain function"

Role: PI Study: To identify functional SNPs in synaptically expressed genes and to examine their association with multiple phenotypic measures of normal brain function in a large population cohort.

NHMRC Project Grant No 455310 Schofield (PI) 1/1/2007-12/31/2009

"Mechanism of signal transduction and receptor activation in ligand gated ion channel receptors"

Role: PI Study: A structure-function study examining the inhibitory glycine receptor.

Past Support

NHMRC Research Fellowship No 157209 Schofield (PI) 1/1/2002 – 12/31/2006

"NHMRC Senior Principal Research Fellowship and Support Enhancement Option"

Role: PI Study: This award was a continuation of a career support fellowship at the full professorial level from the Australian NHMRC to support my full time research salary.

NHMRC Project Grant No 276401 Schofield (PI) 1/1/2004-12/31/2006

"Identification and characterisation of phenotypic modifier genes in familial Alzheimer's disease"

Role: PI Study: Characterised variable clinical phenotypes in AD and resulted in the identification of a novel kinase that modifies AD genes and a new genetic locus that may encode a disease modifying gene.

NHMRC Project Grant No 230802 Schofield (PI) 1/1/2003 – 12/31/2005

"Understanding the molecular basis of bipolar affective disorder"

Role: PI Study: Using linkage, positional cloning and association analysis the project resulted in the successful identification of the cadherin gene FAT as the first positionally-cloned bipolar disorder susceptibility gene.

NHMRC Project Grant No 276403 Schofield (PI) 1/1/2004-12/31/2006

"Molecular determinants of inhibitory synaptic function studied using mutant and transgenic mice"

Role: PI Study: Using various and mutant mouse lines, the electrophysiological nature of synaptic inhibition was examined. Provided evidence for a mechanism of functional compensation in synaptic signaling.



The Nobel Prize in Physiology or Medicine 1993



Press Release

NOBELFÖRSAMLINGEN KAROLINSKA INSTITUTET
THE NOBEL ASSEMBLY AT THE KAROLINSKA INSTITUTE

11 October 1993

The Nobel Assembly at the Karolinska Institute has today decided to award the 1993 Nobel Prize in Physiology or Medicine jointly to

Richard J. Roberts and Phillip A. Sharp

for their discovery of "split genes".

Summary

Our knowledge regarding the genetic material, the genes, has increased dramatically during the last forty years due to achievements in the area of molecular biology. During the first decades, studies on simple organisms, in particular bacteria and bacterial viruses, dominated. A gene was conceived as a continuous segment within the very long double-stranded DNA molecules, the chemical substance of heredity. This simple picture of gene structure completely changed when **Richard J. Roberts and Phillip A. Sharp** in 1977 independently discovered that genes could be discontinuous, that is, a given gene could be present in the genetic material (DNA) as several, well-separated segments. As their experimental model system, both Roberts and Sharp used a common cold-causing virus, called adenovirus, whose genes display important similarities to those in higher organisms. Shortly thereafter it could be shown by several researchers that split genes are frequent in higher organisms, including man.

Roberts' and Sharp's discovery has changed our view on how genes in higher organisms develop during evolution. The discovery also led to the prediction of a new genetic process, namely that of splicing, which is essential for expressing the genetic information. The discovery of split genes has been of fundamental importance for today's basic research in biology, as well as for more medically oriented research concerning the development of cancer and other diseases.

The genetic material

During the last forty years our knowledge of how the genetic material, the genes, governs the basic activities of life has increased dramatically. This is due to progress made within molecular biology, the area in science which explores biological phenomena and structures at the molecular level. Many of the most important discoveries within this area have been awarded a Nobel Prize. Examples include the discovery of how the nucleic acid DNA, the chemical substance of heredity, is built (1962), how the synthesis of nucleic acids takes place (1959), how the activity of genes is regulated (1965) and what the genetic code looks like (1968). This knowledge evolved primarily through studies of simple organisms such as bacteria and viruses infecting bacteria.

The general concept prevailing during the mid 1970s regarding the hereditary material and its function can be summarized as follows. A gene exists as a continuous stretch (segment) within a long, double-stranded DNA molecule. When the gene is activated, its information is copied into a single-stranded RNA molecule, called messenger RNA, which translates the information into a protein (figure 1A).

This simple picture of the sequence of events radically changed through the discovery made in 1977 by **Richard J. Roberts**, working at the Cold Spring Harbor Laboratory on Long Island, New York, and **Phillip A. Sharp**, working at the Massachusetts Institute of Technology in Cambridge, USA. They found that an individual gene can comprise not only one but several DNA segments separated by irrelevant DNA (figure 1B). Such discontinuous genes exist in organisms more complex than those studied earlier.

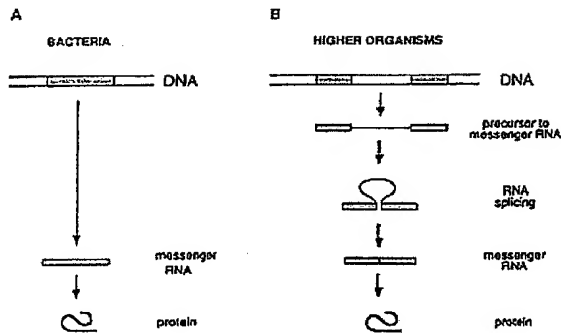


Figure 1: Gene structure and the flow of genetic information in bacteria (A) and higher organisms (B). In bacteria, the genetic information is stored as a continuous segment of DNA, and the messenger RNA can immediately direct the synthesis of the corresponding protein. In higher organisms, the gene is usually split, and the messenger RNA has to be processed by splicing before it can be translated into a protein.

How the discovery was made

Roberts and Sharp were studying the genetic material in adenovirus, a virus causing common cold. This virus infects the cells of higher organisms, and its genome has many properties resembling those of the host cell. At the same time, adenovirus has a simple structure, making it a very valuable experimental model for studying genes and their function in higher organisms. The genome of adenovirus consists of one single long DNA molecule. Roberts' and Sharp's aim was to determine where in the genome different genes were located.

In biochemical experiments it was shown that one end of an adenovirus messenger RNA did not behave as expected. One of several possible explanations was that the DNA segment corresponding to this end was not located in the immediate vicinity of the rest of the gene. To determine where this segment was located on the long DNA molecule, they used electron microscopy. They surprisingly found that a single RNA molecule corresponded to no less than four well-separated segments in the DNA molecule (figure 2). Roberts and Sharp came to the conclusion that the genetic information in the gene was discontinuously organized in the genome, a conclusion that contradicted the commonly held view regarding the structure of genes. The discovery immediately led to intensive research to find out whether this gene structure is present also in other viruses and in ordinary cells. Very soon after the initial discovery, several researchers could show that a discontinuous (or split) gene structure was common – and in fact the most common gene structure in higher organisms.

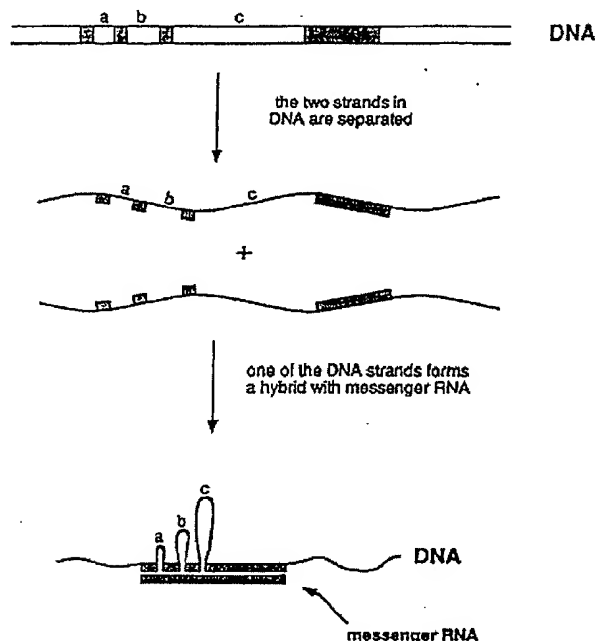


Figure 2: Schematic representation of the experiment that demonstrated that adenovirus DNA contains split genes. The genetic information in the messenger RNA resides in the DNA as four segments, which are separated by three intervening regions (a, b, and c). In the experimentally produced hybrid between one of the DNA strands and the RNA, the intervening sequences in the DNA strand appear as loops, i.e. the corresponding segments lack counterparts in the RNA. The hybrid could be directly visualized in the electron microscope.

The importance of the discovery

A gene may thus consist of several segments, usually termed *exons* separated by intervening DNA, termed *introns*. This knowledge has radically changed our view on how the genetic material has developed during the course of evolution. It has long been considered likely that evolution takes place as the result of the accumulation of minor alterations in the genetic material (mutations) resulting in a gradual change.

As a consequence of the discovery that genes are often split, it seems likely that higher organisms in addition to undergoing mutations may utilize another mechanism to speed up evolution: rearrangement (or shuffling) of gene segments to new functional units. This can take place in the germ cells through crossing-over during pairing of chromosomes. This hypothesis seems even more attractive following the discovery that individual exons in several cases correspond to building modules in proteins, so-called domains, to which specific functions can be attributed. An exon in the genome would thus correspond to a particular subfunction in the protein and the rearrangement of exons could result in a new combination of subfunctions in a protein. This kind of process could drive evolution considerably by rearranging modules with specific functions.

The discovery that genes can consist of two or more segments immediately led to a prediction with both surprising and important consequences. The first RNA product synthesized containing both exons and introns has to be "edited" such that the introns are cut out and the remaining exons are joined together to form a shortened RNA molecule. It has now been established that this process indeed takes place, and we have already accumulated detailed information on its nature. The process is called *splicing* and in higher organisms it represents an additional step in the transfer of information as compared to what usually occurs in lower organisms (figure 1B). The importance of splicing became particularly apparent, when it was found that it is not always the same segments that are identified as exons and are included in the final RNA molecule. In different tissues or developmental stages, the final RNA molecule may be different due to the utilization of alternative exon combinations. Thus, the same DNA region can in many cases determine the structure of many different proteins. The process is called alternative splicing and represents a fundamentally new principle: the genetic message, which gives rise to a particular product, is not definitely established at the stage when the RNA is first synthesized. Instead, it is the splicing pattern that determines the nature of the final product.

Medical aspects

Hereditary diseases are common - their estimated number is today no less than about 5000. Some of them are due to errors in the splicing process. The most studied of such diseases is beta-thalassemia, an anemia prevalent primarily in some Mediterranean countries.

The disease is due to a faulty protein, which forms part of hemoglobin in red blood cells. The protein is called beta-globin. If no or badly functioning beta-globin is made, the life-span of the red blood cells is shortened resulting in anemia. In different patients, small defects in the genetic material have been found, resulting in errors in the splicing process and thus in the synthesis of poorly functioning beta-globin. In the upper part of figure 3 the normal splicing of beta-globin RNA is shown (A). If the globin gene is damaged (marked by an arrow) it may, for example, lead to the formation of a larger than normal exon during splicing (B), or to the formation of a completely new exon (C).

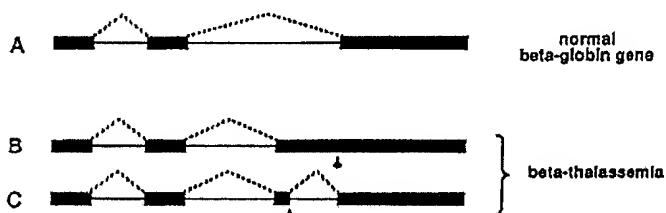


Figure 3: Defective splicing causing beta-thalassemia. A normal beta-globin gene is presented in A, and two mutated genes that result in beta-thalassemia are shown in B and C. Arrows mark the position of point mutations. The interrupted lines denote the segments that are being joined during the splicing process. In the healthy individual, three segments are spliced as shown in A. In one of the thalassemia cases, an unusually long third segment is formed (B), while in the second one, an extra segment is produced (C).

Another example showing the connection between disease and the organisation of the genetic material into exons and introns is chronic myeloid leukemia, a type of cancer of the blood. Characteristic for this disease is the presence in tumor cells of a special, shortened chromosome, called the Philadelphia chromosome, named after the city in which it was discovered. This chromosome has arisen in a white blood cell by fusion of one end of chromosome 22 to one end of chromosome 9. At the break-point, a large portion of a cancer gene has been joined to another gene. Here we are thus dealing with two genes, which are now copied into one single RNA molecule. During the splicing process exons from the two genes are spliced to form an RNA molecule that specifies the synthesis of a new protein, a so-called fusion protein. This new protein gives rise to leukemia.

References

- B. Alberts et al: Molecular Biology of the Cell. Garland, New York, 1989
- P. Chambon: Split Genes. Scientific American **244**, 60-71 (1981)

J.E. Darnell: RNA. Scientific American **253**, 68-78 (1985)

J.E. Darnell et al: Molecular Cell Biology. Scientific American Books. Freeman, New York, 1990

E.H. McConkey: Human Genetics. The Molecular Revolution. Jones and Bartlett, Boston, 1993.

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